Effects of Kaolin Particle Film on Oviposition, Larval Mining, and Infestation of Cotton by Pink Bollworm (Lepidoptera: Gelechiidae)

M. S. SISTERSON,[1] Y. B. LIU,[2] D. L. KERNS,[3] AND B. E. TABASHNIK[1]

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ABSTRACT We tested effects of kaolin particle film on oviposition, larval mining, and infestation of cotton by pink bollworm, *Pectinophora gossypiella* (Saunders) (Lepidoptera: Gelechiidae), in the laboratory, greenhouse, and field. In laboratory choice tests, females laid seven times more eggs on untreated bolls than on bolls treated with kaolin. When neonates were put on bolls in the laboratory, each boll with a treated and untreated half, larvae and mines were found 24 h later on the untreated half but not on the treated half. In oviposition choice tests with whole plants in the greenhouse, females laid four times more eggs on untreated plants than on treated plants and the number of eggs on bolls was five times higher for untreated plants than for treated plants. Kaolin treatments altered the distribution of eggs among plant parts, with untreated bolls receiving a higher percentage than treated bolls, whereas the opposite occurred for petioles. In field tests, treatment with kaolin alone reduced the proportion of bolls infested with pink bollworm, but a mixture of kaolin and the pyrethroid lambda-cyhalothrin was most effective. The results suggest that kaolin particle film may be useful against pink bollworm, particularly in conjunction with other control tactics.

KEY WORDS Kaolin, particle films, *Pectinophora gossypiella,* pink bollworm, cotton, integrated pest management (IPM)

THE PINK BOLLWORM, *Pectinophora gossypiella* (Saunders) (Lepidoptera: Gelechiidae), is a major pest of cotton in the southwestern United States and elsewhere (Ingram 1994, Henneberry and Naranjo 1998). Females lay eggs on or near bolls and larvae feed exclusively within bolls where they are protected from insecticide sprays (Henneberry and Naranjo 1998). As a result, insecticide sprays target adults and several applications are required to maintain control (Henneberry and Naranjo 1998). Despite the success in Arizona of transgenic cotton that produces *Bacillus thuringiensis* (Bt) toxin Cry1Ac, much cotton without Bt is grown in Arizona and in other areas where the pink bollworm is a major pest (Carriere et al. 2001). Indeed, a non-Bt cotton refuge must be maintained when growing Bt cotton in the United States (U.S. EPA 1995). Thus, development of safe and effective alternatives to Bt cotton for managing pink bollworm is desirable.

Kaolin is an environmentally benign, white, nonabrasive, fine-grained, aluminosilicate mineral (Glenn et al. 1999). In several agricultural systems, coating of plants with a kaolin particle film reduces pest population density (e.g., Glenn et al. 1999, Unruh et al. 2000, Puterka et al. 2000). The mechanisms responsible for this reduction vary, but reduced oviposition and feeding on treated plants are most commonly reported (Glenn et al. 1999).

The primary objective of our study was to determine if kaolin particle film deters pink bollworm oviposition and larval mining. To address this objective, we conducted oviposition and larval mining choice tests in the laboratory with bolls and in the greenhouse with whole cotton plants. We also compared effects of kaolin particle film, a pyrethroid, and a combination of both on infestation of bolls by pink bollworm in the field.

Materials and Methods

Insects

In laboratory and greenhouse experiments, we tested the APHIS-S strain of pink bollworm, which has been reared in the laboratory for >20 yr without exposure to insecticides. Larvae were reared in groups of several hundred on wheat germ diet in 500 ml cups (Bartlett and Wolf 1985). Pupae were washed in a 10% bleach solution and put in groups of ≈150 in new 500 ml cups in which adults emerged. The cups were covered with paper towel (Chicopee, Benson, NC), which acted as an oviposition substrate. Honey water solution was provided for food. Eggs were collected every other day. Eggs, larvae, and adults were held at 27°C and 14:10 photoperiod. For the oviposition tests we used 3–7 d old adults as this is the period when oviposition activity is highest (Lingren et al. 1988).

[1] Department of Entomology, University of Arizona, Tucson, AZ 85721 (e-mail: mark_s@ag.arizona.edu).
[2] USDA–ARS, 1636 E. Alisal Street, Salinas, CA 93905.
[3] Yuma Agricultural Center, University of Arizona, Yuma AZ 85364.

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Plants

For laboratory and greenhouse experiments Deltapine 54–15 cotton (Delta and Pine Land Co., Cantre, AL) was grown in a greenhouse. Plants were fertilized once a month with Osmocote (14-14-14) (The Scotts Co., Marysville, OH), Ironite (1–0-0) (Ironite Products Co., Scottsdale, AZ), and Peters Peat-lite Special (15-16-17) (The Scotts Co., Marysville, OH). Plants were used when bolls were ≈2 wk old because bolls of this age receive the most eggs and larval mines (Liu et al. 2002).

Kaolin

The kaolin used in all experiments was Surround WP Crop Protectant (Engelhard Corp., Iselin, NJ) (referred to as kaolin for the remainder of this paper). This product contains 95% kaolin and 5% other ingredients. For laboratory and greenhouse studies we used a 5% slurry of kaolin powder in water.

Laboratory Tests

The laboratory tests examined larval mining and oviposition response to treated and untreated cotton bolls. Cotton bolls were collected from greenhouse grown plants on the morning of each experiment and were transported to the laboratory with the stem of each boll in water. In the laboratory, a 5% kaolin slurry was applied to a randomly selected group of bolls with a small paintbrush. After treated bolls dried, the stems of treated and untreated bolls were inserted into water-soaked foam blocks (Aquafoam, Syndicates Sales Inc., Kokoma, IN) before experiments.

To determine the effect of kaolin on larval mining, we treated half of each cotton boll with kaolin and left the other half untreated. After bolls dried, we put ten neonates on the border between the treated and untreated halves of each boll. Twenty-four hours later, we recorded the number of mines and larvae found in each half of the boll. We completed a total of eight replicates and compared the number of mines and larvae on each half of the bolls using a paired *t*-test.

To evaluate the effect of kaolin on oviposition, we compared the number of eggs laid by females on treated and untreated bolls in screened cages ($23 \times 23 \times 23$ cm). Each cage had four treated bolls, four untreated bolls, and 20 moths. After 24 h, bolls were removed and the number of eggs on each boll was recorded. A total of 22 replicates were completed. The number of eggs laid on treated and untreated bolls was compared using a paired *t*-test.

Greenhouse Tests

Greenhouse tests to evaluate effects of kaolin on oviposition on whole cotton plants were conducted in July 2001. Pots containing three cotton plants in 45 liters of soil were paired based on the size and number of bolls on the plants. For each pair, one set of plants was sprayed with ≈500 ml of 5% kaolin slurry using a hand-held sprayer. After plants dried, each pair of pots was put in a $76 \times 47 \times 112$ cm cage in a greenhouse. Two hundred moths were then released into each cage at 1600–1800 MST. A honey water solution was provided for food. The next morning at 800–1000 MST plants were collected. We recorded the number of eggs laid on bolls, leaf surfaces, petioles, stems, and axils. We also recorded the longitudinal diameter of each boll that received eggs. We conducted a total of eight replicates over 2 d (four each day). Temperature in the greenhouse during the experiment was 20–32°C.

We used paired *t*-tests to determine if treated and untreated plants differed significantly in number of eggs laid overall and on each of five plant parts separately (bolls, leaf surfaces, petioles, stems, and axils). Next, we compared the proportion of eggs laid on each plant part in the two treatments using a paired *t*-test. Understanding how the eggs were distributed among plant parts is of interest because some parts are likely to receive more coverage with kaolin than others.

Because boll size affects oviposition (Liu et al. 2002), we compared the size distribution of bolls that received eggs between treated and untreated plants, using a chi-square test of homogeneity (Steel and Torrie 1980). We also used linear regression to determine the relationship between boll diameter and the number of eggs per boll (log transformed) for treated and untreated plants (SAS Institute 2001). Finally, we used a paired *t*-test to determine if the number of bolls per pot differed between treated and untreated pots.

Field Tests

We tested kaolin in field plots at the Yuma Agricultural Center (Yuma County) using a randomized complete block design (four replicates). Each plot was eight rows wide and 11.5 m long. Rows were 1 m apart with 3 m alleys between plots. The two outside rows of each plot were not treated and were not sampled. Deltapine 54–15 cotton was planted on 18 April 2001 and was grown using standard practices. The field trial included six treatments: (1) an untreated control, (2) kaolin alone, (3) a pyrethroid alone, (4) a mix of kaolin and the pyrethroid, (5) a cyclodiene alone, and (6) a mix of the cyclodiene and the pyrethroid. All treatments were applied using a tractor mounted sprayer. Kaolin was applied at a rate of 67 kg per ha. The pyrethroid was lambda-cyhalothrin (Warrior T, Zeneca Ag Products, West Layfayette, IN), which is currently recommended to control pink bollworm (Ellesworth et al. 1994). It was applied at a rate of 40.5 g of active ingredient per ha. The cyclodiene was endosulfan (Thiodan 3 EC, FMC Corp., Philadelphia, PA) and is recommended for control of *Lygus* bugs and was applied at a rate of 0.70 kg of active ingredient per ha (Ellesworth et al. 1994).

The comparison of kaolin to the pyrethroid is of direct interest, because this pyrethroid is a conventional control for pink bollworm. Alternatively, the cyclodiene treatments were not of direct interest and were included in the field tests for a separate study

Table 1. Effects of kaolin on numbers of eggs laid in the greenhouse choice tests. The number of eggs laid on each plant part was compared using paired *t*-tests

Plant Part	Mean number of eggs per pot ± SEM[a]	
	Treated	Untreated
Bolls	29.3 ± 8.10b	147.1 ± 20.9c
Leaves	5.6 ± 1.2b	18.0 ± 2.8c
Stems	4.4 ± 0.9b	65.4 ± 9.2c
Axils	31.3 ± 4.8b	140.5 ± 17.6c
Petioles	21.4 ± 2.3b	65.4 ± 9.2c
Whole Plant	91.9 ± 12.8b	389.3 ± 46.1c

[a] Pairs within a row that are followed by different letters are significantly different ($\alpha = 0.05$).

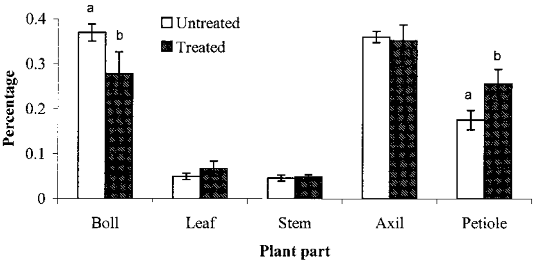

Fig. 1. Percentage of eggs laid on different plant parts in greenhouse choice tests. Mean ± SEM are shown. For each plant part, means followed by different letters are significantly different (paired *t*-test, $\alpha = 0.05$).

examining effects on *Lygus.* Results from the cyclodiene treatments were included in the statistical analysis here because they aided in estimating the block effect and increased power.

Plots receiving kaolin alone and kaolin plus pyrethroid were treated 11 times to maintain coverage for the entire season (15, 25 May; 5, 20, and 29 June; 13, 27 July; 9, 17, 24, and 31 August). Plots treated with the pyrethroid alone, the cyclodiene alone, and the pyrethroid plus cyclodiene were treated only on the last three dates (17, 24, and 31 August).

On each of four dates (20, 24, and 29 August; and 6 September), we collected 100 bolls per plot and determined the proportion of bolls infested with pink bollworm larvae. Sampling dates were timed to occur ≈5–7 d after a pesticide application as this is the time interval used by field scouts. We tested for an overall effect of treatment using MANOVA and then used analysis of variance (ANOVA) to conduct multiple comparison tests [Tukey's least significant difference (LSD)] for each sampling date (SAS Institute 2001).

Results

Laboratory Tests

In choice experiments with half of each boll untreated and half treated with kaolin, mines occurred in the untreated half (3.5 ± 0.7 SEM mines per boll), but not in the treated half ($t = 5.00$, df = 7, $P < 0.01$). Likewise, larvae were found in the untreated half of bolls (1.4 ± 0.4 SEM larvae per boll), but not in the treated half ($t = 3.67$, df = 7, $P = 0.008$). The number of eggs laid per boll was seven times higher for untreated bolls (24.8 ± 3.8 SEM eggs per boll) than for treated bolls (3.4 ± 2.1 SEM eggs per boll) ($t = 6.46$, df = 21, $P < 0.0001$).

Greenhouse Tests

In greenhouse oviposition choice tests with whole cotton plants in pots, the number of eggs laid per pot was four times greater for untreated plants than for treated plants (Table 1). Likewise, the number of eggs laid per pot was significantly greater for untreated plants than for treated plants for each of five plant parts (bolls, leaves, stems, axils, and petioles) considered separately (Table 1). Similar to the seven-fold difference observed between untreated and treated bolls in laboratory tests (above), females laid five times more eggs on untreated bolls than on treated bolls in greenhouse tests.

The distribution of eggs within plants differed significantly between treated and untreated plants for bolls and petioles, but not for leaves, stems, and axils (Fig. 1). Of the total eggs laid on each type of plant, the percentage laid on bolls was significantly higher for untreated plants (36%) than for untreated plants (27%) ($t = 2.64$, df = 7, $P = 0.02$). Conversely, the percentage of eggs laid on petioles was higher for treated plants (26%) than for untreated plants (18%) ($t = 2.32$, df = 7, $P = 0.03$).

The size distribution of bolls that received eggs did not differ between the two treatments ($\chi^2 = 10.21$, df = 9, $P > 0.25$) (Fig. 2A). For untreated plants there was a significant increase in the number of eggs deposited per boll with boll diameter ($F = 8.33$, df = 1, 180, $P = 0.004$, $r^2 = 0.04$). A similar trend was observed for the bolls on kaolin treated plants, but was not significant ($F = 2.46$, df = 1, 50, $P = 0.12$, $r^2 = 0.05$). However, the slope of the regression line for the treated and untreated plants was the same. The common slope suggests a similar response to bolls size, but an overall decrease in oviposition rate (Fig. 2B).

The number of bolls per plant did not differ significantly between untreated (46.4 ± 6.6) and treated plants (44.9 ± 3.4) ($t = 0.33$, df = 7, $P = 0.75$). Thus, differences between treatments were not caused by a difference in the number of bolls per plant.

Field Tests

The MANOVA indicates an overall effect of treatment on proportion of bolls infested by pink bollworm (Wilks' Lambda, $F = 1.92$, df = 24, 38, $P < 0.04$). Multiple comparison tests show significant variation on all sampling dates except for the first one (Fig. 3). In general, the combination of the pyrethroid and kaolin provided the best control, followed by kaolin alone, and the pyrethroid alone (Fig. 3). As expected the cyclodiene treatment had little effect on pink bollworm numbers and was significantly different from the untreated control only on a single sampling date (24 August). Likewise, the effects of the cyclo-

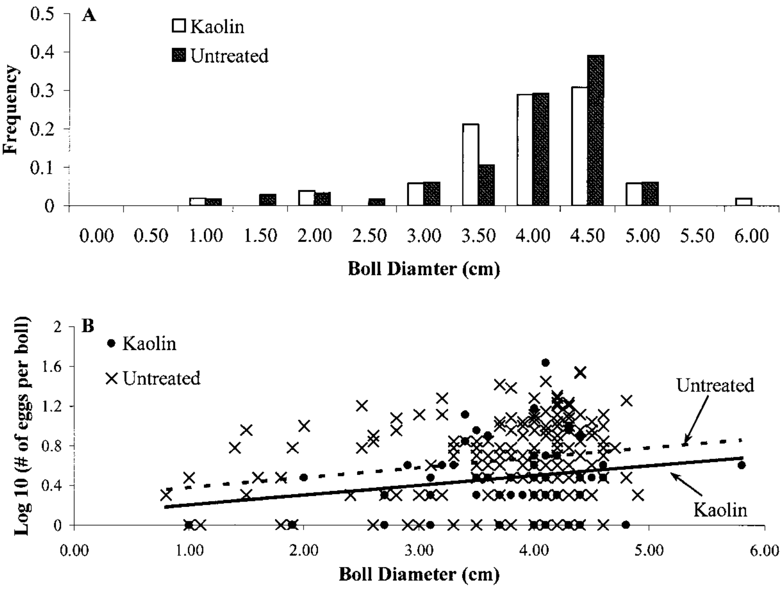

Fig. 2. Response of moths to treated and untreated bolls on whole plants in the greenhouse study. (A) Size distribution of bolls that received eggs in the two treatments. The distributions for the treated and untreated bolls were not significantly different. (B) Regression of boll diameter versus the number of eggs laid on that boll (log 10 transformed). The regression was significant for the bolls on untreated plants ($y = 0.10x + 0.28$, $r^2 = 0.04$), but not for the bolls on treated plants ($y = 0.10x + 0.10$, $r^2 = 0.05$).

diene plus pyrethroid treatment did not differ significantly from the pyrethroid alone.

Discussion

Our laboratory and greenhouse results show that kaolin particle film deterred pink bollworm oviposition and larval mining. No larval mines or larvae were found in the kaolin-treated halves of bolls in laboratory experiments. However, kaolin treatments did not completely deter oviposition. In the laboratory, untreated bolls received seven times more eggs than treated bolls, while in the greenhouse, untreated plants received four times more eggs than treated plants.

Treatment with kaolin not only reduced the number of eggs laid per boll or plant, it also altered the relative amount of eggs laid on different plant parts. In particular, the percentage of eggs on bolls was higher for untreated plants than treated plants, but the re-

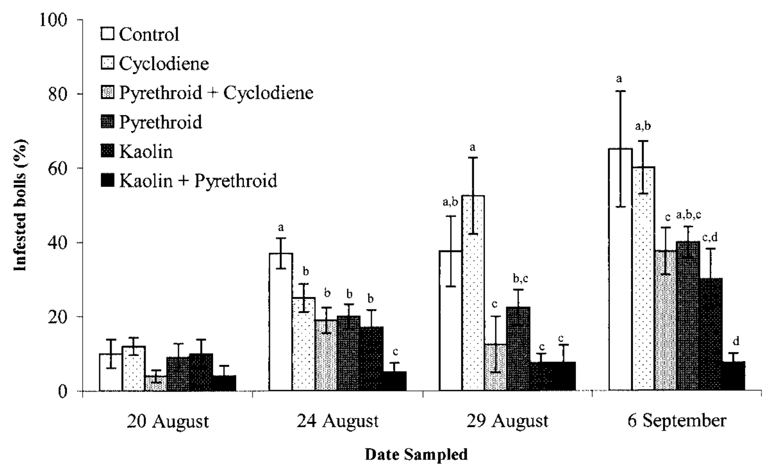

Fig. 3. Percentage of infested bolls on each sampling date during the field study. While, the pyrethroid is a recommended control for pink bollworm, the cyclodiene is not. Thus, we expect the level of control for the cyclodiene to be equal to the control. Mean ± SEM are shown. For each date, means followed by different letters are significantly different (Tukey's LSD, $\alpha = 0.05$).

verse was true for petioles (Fig. 1). For petioles of treated plants, eggs were laid only on the underside, an area that received minimal coverage with the particle film. This suggests that oviposition on a particular part of the plant depended on particle film coverage of that part of the plant.

With regards to oviposition on bolls, the greenhouse study demonstrated that there was no change in the size distribution of attacked bolls (Fig. 2A). Additionally, larger bolls generally received more eggs. Although, this trend was only significant for the untreated control, the common slope of the two regression analyses suggests a similar response to boll size (Fig. 2B). These results indicate that the response of pink bollworm moths to kaolin treated bolls of different sizes was not altered, but that there was an overall suppression of oviposition.

Kaolin treatments also reduced pink bollworm infestation in cotton bolls in field tests. The level of control achieved by kaolin was equal to or better than that of a currently recommended pyrethroid. However, a combination of the pyrethroid and kaolin provided the greatest level of control and was the only treatment that held pink bollworm below or near the economic action level [5–15% of bolls infested, depending on geographic location (Ellsworth et al. 1994)].

The level of suppression achieved by kaolin in the field was somewhat lower than the reduction in oviposition and mining seen in laboratory and greenhouse experiments. In the field, the effect of the kaolin treatment varied from zero- to fivefold reduction in the proportion of infested bolls. In particular, only a twofold reduction in the proportion of bolls infested occurred on the final sampling date (6 September) when pink bollworm density was highest (Fig. 2). This contrasts with the four- to sevenfold reduction in oviposition observed in the laboratory and greenhouse experiments.

Lower initial coverage and decreases in coverage with time may have contributed to the weaker effects of kaolin in the field. Bolls in the laboratory were treated using a paintbrush and plants in the greenhouse were treated using a hand held sprayer. Coverage achieved with these methods was probably greater than the coverage achieved with a tractor-mounted sprayer in the field. While laboratory and greenhouse tests examined responses during the first day after treatment with kaolin, 7–15 d passed between kaolin treatments in the field. Because kaolin particle film wears off with time (Unruh et al. 2000) and field conditions may accelerate this effect, the different time scales in our experiment might have been an important factor.

Aside from effects on pink bollworm, kaolin treatments may provide additional benefits for cotton production. For example, kaolin treatments might deter other insect pests from ovipositing and feeding on cotton. Several of these pests, such as whiteflies in the genera *Bemisia* and *Trialeurodes*, are known disease vectors (Butler and Henneberry 1994). Prevention of plant colonization by disease vectors is likely to have high returns. Kaolin has also been hypothesized to control fungal and bacterial plant pathogens by preventing the formation of a liquid film on the surface of leaves (Glenn et al. 1999). Finally, kaolin may also have agronomic benefits. A study on the Acala SJ-2 variety of cotton in Israel suggests that kaolin treatment can increase yield and flower production by decreasing transpiration (Moreshet et al. 1979). Additional work assessing these and other potential benefits will be needed to more fully determine the economic value of kaolin treatments for cotton production.

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References Cited

Bartlett, A. C., and W. W. Wolf. 1985. *Pectinophora gossypiella. In* R. F. Moore and P. Singh [eds.], Handbook of insect rearing, vol. 2. Elsevier, Amsterdam, pp. 415–430.

Butler, G. D., and T. J. Henneberry. 1994. *Bemisia* and *Trialeurodes* (Hemiptera: Aleyrodida), pp 325–352. *In* G. A. Matthews, J. P. Tunstall [eds.]. Insect Pest of Cotton. Cab International, Cambridge, United Kingdom.

Carriere, Y., Dennehy, T. J., Pedersen, B., Haller, S., Ellers-Kirk, C., Antilla, L., Liu, Y. B., Willott, E., and B. E. Tabashnik. 2001. Large-scale management of insect resistance to transgenic cotton in Arizona: can insecticidal crops be sustained? J. Econ. Entomol. 94: 315–325.

Ellsworth, P., Moore, L., Watson, T. F., and T. Dennehy. 1994. Insect pest management for cotton. Cooperative Extension, College of Agriculture, University of Arizona, Tucson. Publication 194022.

[EPA] U.S. Environmental Protection Agency. 1995. Pesticide fact sheet: *Bacillus thuringiensis* subspecies Cry1Ac delta-endotoxin and its controlling sequence as expected in cotton. Office of Pesticide Programs. Washington, DC.

Glenn, D. M., Puterka, G. J., Vanderzwet, T., Byers, R. E., and C. Feldhake. 1999. Hydrophobic particle films: a new paradigm for suppression of arthropod pests and plant diseases. J. Econ. Entomol. 92: 759–771.

Henneberry, T. J., and S. E. Naranjo. 1998. Integrated management approaches for pink bollworm in the southwestern United States. IPM Rev. 3: 31–52.

Ingram, W. R. 1994. Pectinophora (Lepidoptera: Gelechiidae), pp. 107–148. *In* G. A. Mathews and J. P. Tunstall [eds.], Insect pests of cotton. CAB, Wallingford, United Kingdom.

Lingren, P. D., Warner, W. B., and T. J. Henneberry. 1988. Influence of delayed mating on egg production, egg viability, mating, and longevity of female pink bollworm (Lepidoptera: Gelechiidae). Environ. Entomol. 17: 86–89.

Liu, Y. B., Tabashnik, B. E., Dennehy, T. J., Carriere, Y., Sims, M. A., and S. K. Meyer. 2002. Oviposition on and mining in bolls of Bt and non-Bt cotton by resistant and susceptible pink bollworm (Lepidoptera: Gelechiidae). J. Econ. Entomol. 95: 143–148.

Moreshet, S., Cohen, Y., and M. Fuchs. 1979. Effect of increasing foliage reflectance on yield, growth, and physiological behavior of a dryland cotton crop. Crop Sci. 19: 863–868.

Purteka, G. J., Glenn, D. M., Sekutowski, D. G., Unruh, T. R., and S. K. Jones. 2000. Progress toward a liquid formulation of particle films for insect and disease control in pear. Environ. Entomol. 29: 329–339.

SAS Institute. 2001. JMP statistics and graphics guide. Cary, NC.

Steel, R.G.D., and J. H. Torrie. 1980. Principles and procedures of statistics: a biometrical approach. McGraw-Hill Inc., New York.

Unruh, T. R., Knight, A. L., Upton, J., Glenn, D. M., and G. J. Purteka. 2000. Particle-film for suppression of the codling moth in apple and pear orchards. J. Econ. Entomol. 93: 737–743.

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